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Synopsis.

There are now at our disposal many new and wonderful antibiotic drugs. We are just beginning, however, to learn of their potentialities and of the means to use them to the best advantage.

A review of all the present day literature favoured the use of the disc method of testing for bacterial sensitivity to these agents. From no source could it be learned with certainty as to whether the concentrations of antibiotic used on the discs for in vitro testing had any relationship to the clinical effectiveness of the drug when administered to a patient suffering from a bacterial infection.

It was therefore decided, that a study of a large number of case histories of patients whose bacterial invaders had been subjected to the disc method of sensitivity testing, should be made in order to determine the usefulness or otherwise of the test.

This task was carried out during the 1953-54 academic year at the Provincial Laboratory of the University of Alberta.

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THE UNIVERSITY OF ALBERTA

ANTIBIOTICS
SENSITIVITY TESTS AND THEIR CLINICAL APPLICABILITY

A DISSERTATION

SUBMITTED TO THE SCHOOL OF MEDICINE

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

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by

J.D.MUNROE

EDMONTON, ALBERTA

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The Definition of Antibiotics.

It was Waksman in I942 who proposed the use of the term "antibiotics" to define those chemical substances of microbial origin that possess antimicrobial activity. The term has been enlarged to include not only chemical substances of microbial origin,but those of plant and even animal tissues as well. (I)

The Historical Background.

It is of no little interest to briefly review the events that led up to the development of modern antibiotic therapy.

The first antibiotic was discovered nearly five hundred years ago. It appears from legend that a Peruvian Indian had become lost in the jungle and was stricken with fever. He collapsed beside a little pool of water into which a cinchona tree had fallen. He drank the bitter water to quench his thirst and awoke after a troubled sleep to find that his fever had left him. We do not know for certain if this story is true but there is no doubt that the Countess of Chincon was cured of a tertian fever while living with her husband the Viceroy at Lima by the use of cinchona bark. It was she who in I640 introduced quinine to Europe. (2)

From this incident we pass on nearly three hundred years to I903 when Ehrlich along with the Japanese bacteriologist Shiga discovered that trypan red,abenzidine dye would cure laboratory animals infected with trypanasomes. It remained for Ehrlichs pupil Roehl to complete this work producing Bayer 205 (germanin) the best practical remedy for sleeping

sickness yet discovered.

Ehrlichs next discovery was due to his genius in correlating two new advances. The first was that of Thomas and Breinl in Liverpool who had found the organic derivative of arsenic acid, long known by the trade name "Atoxyl", to be a potent remedy for experimental trypanosomiasis in the laboratory and secondly the knowledge acquired in 1905 that syphilis was due to infection by a spirochete. He and his coworkers arrived by 1909, through many stages, at the arsenocompound which they called "Salversan". They demonstrated its outstanding activity against spirochaetal infections, and thus for the treatment of syphilis. Chemotherapy therewith attained one of its greatest triumphs.

During the next quarter century major successes were few but to those living in tropical regions they were indeed of vital interest. Bayer's Elberfield laboratories produced pamaquine and atebrin in 1926 and 1930. Atebrin became of critical importance during World War II when, manufactured in Allied countries under the name mepacrine, it replaced quinine which the Japanese made unobtainable by their conquest of South East Asia. It was in this period that the treatment of Leishmania infections with organic antimony compounds such as Urea stibamine was successfully introduced.

The chemotherapy of bacterial infections began unobtrusively on Christmas day 1932 when the first German patent for prontosil was registered. The results of administering this drug seemed however too good to be true and it was not until February 1935 that Gerhard Domaghs' epoch making com-

munication "in Beitrag zur Chemotherapie der bakteriellen Infektionen" appeared in the Deutsche Medizinische Wochenschrift. Soon there followed the demonstration by Trefouel and his colleagues in the Pasteur Institute that the much simpler moiety of prontosil, sulphanilamide, was the antibacterial agent. The result of a trial in 1936 by Colebrook and his team in London using both prontosil and sulphanilamide on a comparable series of cases suffering from streptococcal septicaemia, put the importance of the discovery beyond question.

In 1938 the development of the series of sulphanilamide derivatives began. Sulphapyridine, sulphathiazole, sulphaguanidine, sulphadiazine, sulphasuxidine, and the rest, each with its special advantages or its more specific value for certain types of infection quickly became available.

Before the possibilities of the sulphanilamide series had been fully developed, another form of therapy against bacterial infections became available from a quarter widely separated from the synthetic approach envisaged by Ehrlich and so far adopted by his successors. The story has so often been told that it need not be repeated here in detail of Alexander Fleming's discovery of penicillin through the action of a mould contaminant on colonies of staphylococci, and his partial exploration of its properties in 1929. It was the new initiative of Florey, Chain and their co-workers ten years later which led to the purification of penicillin and the demonstration of its outstanding practical value as a therapeutic agent. This joint achievement with all that flowed from it,

seems certain to rank as one of the principal events in the history of therapeutic research and its applications. The anticipation of battle casualties had much to do with the rapidity of the developement from laboratory experiment to large scale production.

Penicillin soon proved to have therapeutic applications, first to problems of infection to which war gave special prominence and then to those commoner ungencies of peace,far beyond the range of any which might have been predicted. It was soon found to be an effective remedy for syphilis as well as for a wide range of bacterial infections. Its actions extensively supplemented as well as overlapped those of the sulphanilamide series and even of salversan. There were still bacterial infections,however,particularly among those due to the gram negative and acid fast bacteria,with which no agent so far discovered could deal adequately. These gaps were soon filled in through the rapid exploration of the therapeutic possibilities of a large series of other antibiotic products, to which the brilliant results obtained with penicillin had given so effective a stimulus. What has been achieved in this direction with streptomycin,chloramphenicol,aureomycin and terramycin is a matter of history still in the making and will be discussed in the pages to follow.

The Use of Antibiotics and their Relation to Organismal Sensitivity.

While we are fortunate to be living in that period of time when antibiotics have been brought into general use, these substances were known to the Chinese two thousand five hundred years ago. They were aware of the benefits of moulded soy bean curd as a treatment applied to carbuncles,boils,and like infections. They used this form of treatment as standard proceedure in these conditions. In addition we know that the French Canadian habitant used,and in certain regions is still using mouldy bread as a dressing for wounds.

We should now consider the antibiotics presently available and the uses to which they can be allocated. This may best be done in the chronological order of their appearance.

Tyrothricin.

In the past the soil had always been considered as a reservoir of pathogenic bacteria for the obvious reason that waste matter and bodies are being continually deposited on or in it. It was not until the nineteenth century when bacteriology had emerged as a science that a search was made for known pathogens in soil. The searchers soon found that such organisms do not long survive in normal soil. Proceeding further it was discovered that the chances of survival were much better in sterilized soil and that some organisms which do not survive in natural soil actually grow well in sterilized soil. This gave rise to the idea that microorganisms in

normal unsterilized soil tend to prevent the growth of introduced foreign pathogens.Indeed,with the exception of the microorganisms causing anthrax,tetanus,gas gangrene,undulant fever,and typhoid fever,most pathogens do not survive more than a few days in soil. They perish either because of the failure to obtain proper nutrition or because of their inability to compete with other organisms or as the result of actual antibiosis.

The hypothesis that the destruction of pathogens in soil is caused from the production of antibiotic substances by other microorganisms which are antagonistic to the pathogens and that development of the nonpathogens might be stimulated by the presence of appropriate pathogens,prompted R.J.Dubos to institute his technique of bacterial enrichment of the soil by the artificial addition of cultures of the microorganisms against which an antibiotic was desired. This resulted in the isolation of the aerobic spore forming bacterium later classified as Bacillus brevis which produces the antibiotic tyrothricin.

Dubos and his co-workers investigated their find and learned that it was made up of two main constituents,gramicidin and tyrocidine. Gramicidin is,for all practical purposes the active principle of tyrothricin. It is unfortunate that both gramicidin and tyrocidine are toxic when administered parenterally.In addition both are injurious to red and white corpuscles.

Tyrothricin is fairly active against most gram positive cocci in vitro. It has,however certain uses when applied top-

-ically in such conditions as bovine mastitis,and in localized infections that are accessible to direct irrigation.

In spite of the therapeutic limitations of tyrothricin it proved an excellent example of the proper development of an antibiotic. The findings in this broad study served not only as a model for subsequent investigations in an effort to evaluate new and different antibiotics but stimulated a renewed interest in the search for antimicrobial substances produced by living organisms. It marked the beginning of the antibiotic age.

Penicillin.

The real contribution made be Fleming was his recognition of the fact that an appropriate strain of Penicillium grown on a simple medium can produce a specific substance, which he designated penicillin,and that this substance can be extracted from the medium,and that it is endowed with selective antibacterial activity.It was the tenacious work of Florey and Chain that is most responsible for the eventual development of this drug. It was just before the outbreak of World War II when Florey and Chain began their first experimental work on penicillin.When England entered the war in September I939 penicillin was still a laboratory curiosity, produced by certain penicillium strains and then inconsistently. The great potentialities of this drug were realized after the first human trials carried out in England in I94I. The cases chosen were staphylococcal and streptococcal septicaemias which had been given up as hopeless.

Due to the situation in Britain it was impossible for the government to accept the responsibility for the necessary research that would put penicillin on a production basis. Florey and Heatley therefore went to the United States in the summer of I94I and told of the results of their studies to American industry and the Federal Department of Agriculture.

In I94I there was not enough penicillin in the United States to treat a single case.In I942 there were probably insufficient quantities to treat a hundred cases,but by September I943 there was enough to satisfy the demands of the Armed Forces of the Allies. It is now produced at the rate of over twenty-one tons a month.

Penicillin is produced by the mould Penicillium chrysogenum strain Q-I76. There are six naturally occuring penicillins,F, G, X, dihydro F, K,and O.Penicillin O has been found useful in instances when the patient is sensitive to penicillin G. Penicillin G,however ,probably constitutes more than 90% of present production and has been found to be most satisfactory except in the specific situation mentioned above when penicillin O is to be preferred.

Penicillin is primarily active against the gram positive group of organisms. Pneumococci,streptococci,staphylococci, certain of the clostridia,and lactobacilli are all suseptible to the action of the drug. In the gram negative group,meningococci and gonococci are sensitive but H.influenzae,E.coli, S.typhosa,S.paratyphi,S.enteritidis,P.aeruginosa and certain Monilia strains are resistant to its action.

The resistance of certain of the gram negative organisms

is associated with their ability to produce penicillinase,an enzyme which inactivates penicillin. A relatively large number of organisms including the fungi have been shown to have the capacity to produce this substance. Penicillinase is of value in sterility test procedures and in blood culture studies where it is essential to inactivate penicillin prior to attempting isolation of suspected causitive organisms.

It has marked treponemacidal activity and has become the drug of choice in the treatment of syphilis.

The antibacterial activity of penicillin in vitro is not affected by the presence of blood and serum.

Following experience with the sulphonamides,during which resistant strains of organisms became a serious problem in their clinical use, it was anticipated that a similar situation would arise with the extended use of penicillin. With the exception of the staphylococci,the development of strains of organisms resistant to penicillin has not become too serious a problem. In general,however,the staphylococci are unique among the organisms sensitive to penicillin in that usually they acquire resistance with some facility and tend to maintain a degree,if not all of their resistance on sub culture. It is apparent also from a review of the literature that staphylococci also acquire resistance from contact with penicillin in man.This has been demonstrated rather conclusively by Blair et al (3),during the local and parenteral treatment of chronic osteomyelitis. In staphylococcic infections such as osteomyelitis and subacute bacterial endocarditis which require prolonged treatment with penicillin it

it may be anticipated that unless the infection is eliminated relatively promptly,resistant strains will develope. There is a substantial number of strains of staphylococci resistant to penicillin now and the proportion of these resistant strains to sensitive ones will undoubtedly increase as time goes on.

Penicillin may either be bactericidal or bacteriostatic for microorganisms suseptible to it. This is dependent on the concentration at the site of infection. The exact mechanism of the action of penicillin on suseptible microorganisms has not yet been clearly explained butit does appear that,as with the sulphonamide drugs,its antagonistic action takes place predominantly while the organisms are actively growing and multiplying. In the clinical application of penicillin,where facilities are availableand particularly in infections that are apt to become troublesome,it is important that the causitive organism be identified and their in vitro sensitivity determined as early as possible. For severe and chronic infections this is mandatory not only at the start but at periodic intervals during therapy. This is of particular importance in infections which become resistant to treatment.Such determinations,while not infallible as a guide to therapy, are highly desireable and necessary for the intelligent management of any severe infection.

Pharmacology-toxicity.

The lack of any toxicity has been one of the greatest advantages that penicillin has over the sulphonamide group. In cases of sub acute bacterial endocarditis as much as one hundred million units have been given daily.

Nervous tissue however is vulnerable to the drug when it is directly applied to it unless the dose is small i.e. in the region of five to ten thousand units. The use of penicillin lozenges is liable to lead to glossitis .

One of the serious reactions to penicillin in man is the Herxheimer reaction in syphilis. This reaction has followed the use of penicillin in both early and late syphilis. Alarming manifestations in cases of cardiovascular and neurosyphilis have been reported. Fortunately,this type of reaction occurs infrequently.

Of increasing importance because of their possible universal occurrence are the allergic manifestations which may be observed following the use of penicillin. It was once thought that the impurities which were part of the early preparations were the cause of this response,but it is now known byond doubt that the pure crystalline drug itself is quite capable of causing an allergic reaction in man. Following a report of a single case of hypersensitivity of the tuberculin type to crystalline penicillin in an individual who had no previous contact with this drug,Rostenberg and Welch (4) showed that of 144 persons tested 5 per cent exhibited a positive reaction of the tuberculin type,despite the fact that none of these indivlduals had had previous contact with penicillin. In addition to those individuals who are sensitive on primary contact with the drug,there is a group of probably 10% of the population that may become sensitive following brief or prolonged treatment. Certain individuals become sen-

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sitive following a few injections,others require many. Of the IO per cent of the population who may be prone to develop sensitivity it may be assumed from current literature that from 3 to 6 per cent are now sensitive to the crystalline material.

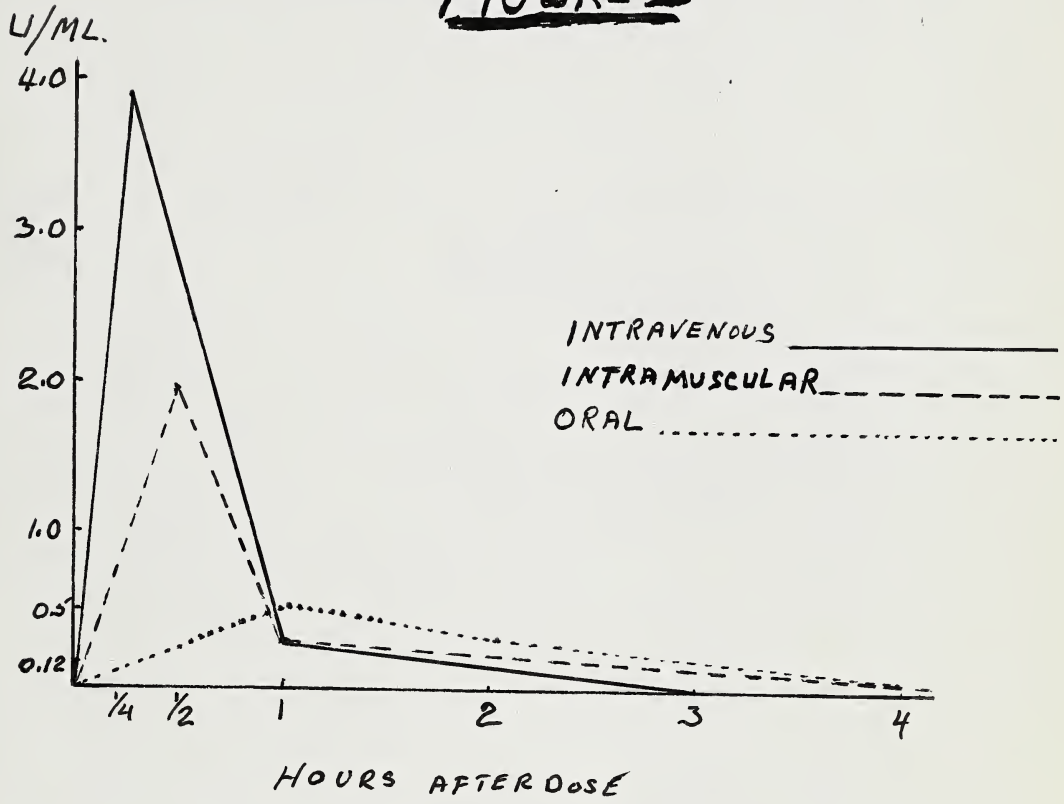
Probably the most common reaction to penicillin is urticaria. This may vary from a single or a few transient erythematous wheals to the diffuse massive plaques of angioneurotic oedema which may be localized on the hands,lips,eyelids, vulva,or larnyx. At times a condition similar to serum sickness may be observed. These reactions may occur from seven to ten days after penicillin therapy has been stoped.

Absorption and excretion.

Since the first clinical trials were made it has been customary to consider that a maintained plasma concentration of 0.03 units per ml. or better is satisfactory for most penicillin suseptible infections other than the most severe.This was an arbitrary figure and it was chosen primarily because of the limitations of bioassay methods utilized for determining blood concentrations of penicillin in man. Obviously some bacterial strains are inhibited by lower concentrations of the drug,while others require higher serum concentrations to obtain the desired therapeutic result. While a maintained plasma penicillin concentration rather than an intermittent one is presently considered most desireable,there is evidence that intermittent or discontinuous therapy may be equally effective in some instances.

The fate of 100,000 units of crystalline penicillin given by the intravenous, intramuscular, and oral routes is shown on the accompanying graph.

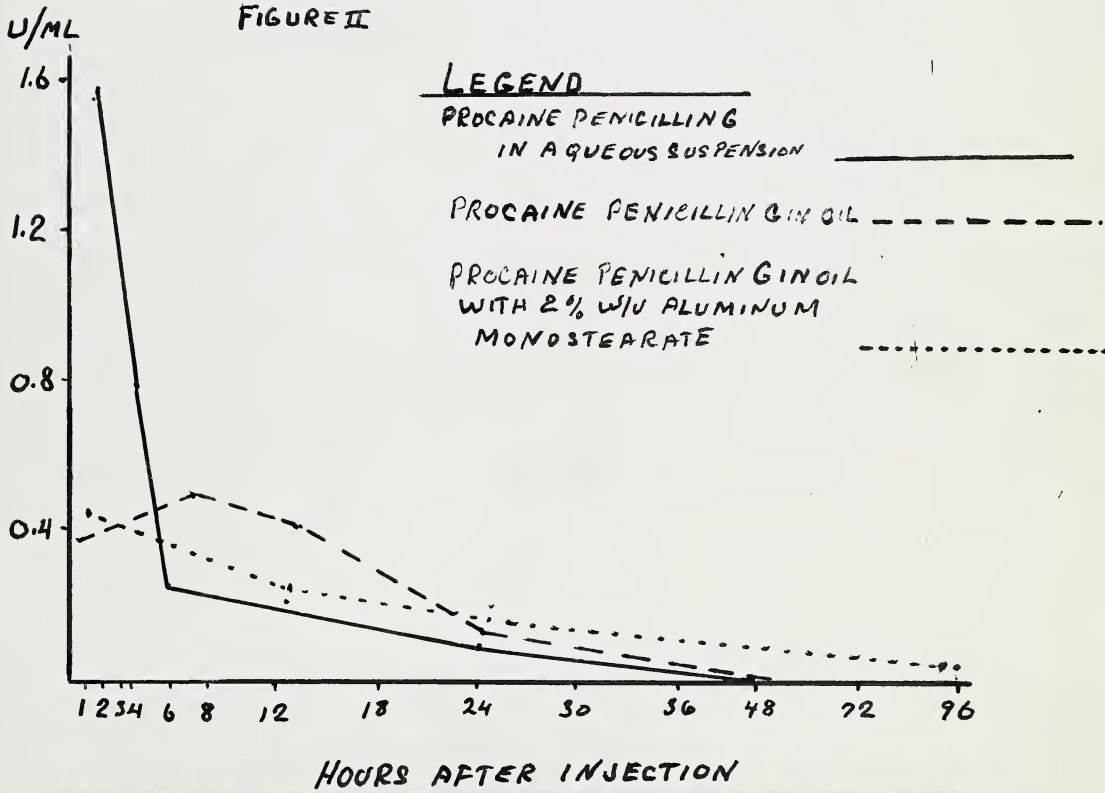
FIGURE I



RELATIVE BLOOD SERUM CONCENTRATIONS OF PENICILLIN FOLLOWING THE INTRAVENOUS, INTRAMUSCULAR OR ORAL ADMINISTRATION OF 100,000 UNITS OF CRYSTALLINE SODIUM PENICILLIN G.

It is obvious that by any of the above routes crystalline penicillin remains in the blood stream for too short an interval and it was not until the drug was combined with oil and aluminum monostearate that maintained plasma levels could be obtained following intramuscular injection.

FIGURE II



AVERAGE BLOOD SERUM PENICILLIN CONCENTRATIONS FOLLOWING THE INJECTION OF 300,000 UNITS OF PROCAINE PENICILLIN IN AQUEOUS SUSPENSION:IN OIL OR IN OIL PLUS 2% w/v ALUMINUM MONOSTEARATE.

The oral route is effective for the treatment of many infections, provided that sufficiently large doses are given one half hour before or two hours after meal times. It is generally accepted that five times as much penicillin is required orally as intramuscularly to obtain comparable plasma concentrations.

Diffusion of penicillin from the circulation into the tissues depends, of course on the plasma concentrations, and will proceed as long as the concentration of diffusible penicillin in the plasma exceeds that in the tissues. The drug diffuses readily from maternal to foetal blood and into the ascitic fluid. Some diffusion also occurs into the pleural pericardial, and synovial fluids and bile. Very little of the drug penetrates into abscess cavities, brain and nerve tissue bone, chambers of the eye or reaches sweat, saliva and tears or maternal milk. Except where the meninges are acutely inflamed, very little diffusion into the cerebrospinal fluid will occur unless very large doses are administered.

Streptomycin and Dihydrostreptomycin.

It was obvious early in the investigation of penicillin that it would not be useful in gram negative infections. Waksman in 1939 attempted to find a substance which would fill this gap. He had been interested in soil bacteriology for many years and chose the actinomyces group of organisms as the ones from which an antibiotic effective against the gram negative group most probably could be found. While he learned that many actinomyces possess antimicrobial substances they were, as a rule either too toxic to use in vivo or inactive when administered to the living animal. It was therefore not until 1944 that the discovery of streptomycin was announced by Waksman, Bugie and Schatz (5).

In streptomycin they believed that they had an antibiotic active against the gram negative group of organisms with a relatively low toxicity. This drug had a special interest in that it had been found to be active against the mycobacteria. It is unfortunate that early hopes for streptomycin have been the cause of much disappointment. Organisms quickly became resistant to its use but it has found a place in the treatment of tuberculosis.

Studies of streptomycin by industrial scientists led to the development of dihydrostreptomycin formed by the catalytic hydrogenation of streptomycin. Dihydrostreptomycin is quite similar to streptomycin in its antibacterial activity although against certain organisms of the gram negative group it appears to be less effective. The tubercle bacillus however appears to be as sensitive to dihydrostreptomycin as to

streptomycin. Unfortunately, resistant organisms develop as rapidly to one as to the other and also organisms resistant to streptomycin are resistant to dihydrostreptomycin as well.

In contrast to penicillin which has for all practical purposes, no toxicity, streptomycin when taken over relatively long periods of time and in certain doses, causes a marked toxic effect exemplified by damage to the eighth cranial nerve with consequent vestibular and auditory damage. It was hoped that dihydrostreptomycin would not be as toxic and at first this seemed to be the case. Reports soon appeared however indicating that there was little advantage in the use of this preparation.

In the treatment of tuberculosis two problems exist with the use of streptomycin and dihydrostreptomycin:(a) the toxicity of either drug may become quite evident in the prolonged treatment required for this disease and (b) after relatively short periods of treatment the tubercle bacillus develops resistance rapidly to either drug. In addition the lesions of tuberculosis are usually fibrotic and to reach them in adequate dosage it is obvious that the drug should be given in large doses (as is penicillin in bacterial endocarditis) if it is going to have any effect. This is not possible with streptomycin or dihydrostreptomycin. Very recently it has been discovered that by combining streptomycin or dihydrostreptomycin with para-aminosalicylic acid the development of resistant tubercle bacilli is less apparent and the treatment can be given over a longer period.

The use of streptomycin with other chemotherapeutic agents such as penicillin is of advantage in diseases other than tuberculosis. In combination less streptomycin is necessary and there is less chance therefore of toxic reactions and in addition the development of resistant organisms is delayed.

In spite of the fact that streptomycin is active against both gram positive and gram negative bacteria,its use in infections caused by gram positive organisms has not been extensive due largely to the efficacy of penicillin in these types of infections. Furthermore,although streptomycin was used fairly extensively in gram negative infections when it first became available in quantity,the relative ease with which resistant organisms developed mitigated against its use. Indeed, gram negative organisms in general develop resistance so rapidly that unless the infection is eliminated promptly on initial treatment,it can become a serious problem for the clinician. With the advent of the three broad spectrum antibiotics,aureomycin,chloramphenicol,and terramycin,all of which are active against gram negative organismsthe problem of treating infections by these organisms has become less acute.During the past year it has become apparent that streptomycin alone is used less and less in the treatment of infections caused by gram negative organisms and its major use the treatment of tuberculosis has expanded.

The early work of Waksman et al with streptomycin demonstrated that it might prove even more liable to stimulate resistance than the sulphonamides. This has now been confirmed.

Of considerable importance to the clinician are the studies

of Finland et al(6) who reported early in the history of this drug that gram negative organisms causing urinary tract infections developed a high degree of resistance to streptomycin during treatment. In a series of twelve cases on infection with various gram negative organisms treated with streptomycin eight failed to show any benefit. Of greater significance however is the fact that organisms on isolation from five of these cases were found to have acquired more than a four thousand fold resistance to the drug within a period of forty-eight hours.

Not only is it possible to demonstrate the development of organisms resistant to streptomycin but in addition certain organisms have been shown to be streptomycin dependent. Miller and Bohnhoff (7) have reported the isolation of variants of meningococci not only resistant to this drug but capable of growing only in its presence.Other bacterial species including S.aureus,E.coli,P.aeruginosa,K.pneumoniae,P.morgani and the tubercle bacillus have been shown to develop variants capable of growing only in the presence of streptomycin.

A streptomycinase similar in its action to penicillinase has not been found,although a large number of microorganisms have been tested for their ability to produce this substance. Streptomycin is inactivated by cysteine,hydroxylamine,and semicarbazide. This fact has been used in sterility testing and blood culture studies where it is necessary to inactivate the drug in order to demonstrate the presence of contaminating or infecting organisms. Dihydrostreptomycin is more stable and is not inactivated by the above substances.

The fact that tubercle bacilli become resistant to streptomycin during prolonged therapy is a serious one, and may eventually become a public health problem. The transfer of streptomycin resistant strains of tubercle bacilli to previously uninfected persons was reported in I948. Since streptomycin is used today extensively in the treatment of tuberculosis and the complications of tuberculosis it is not unlikely that eventually we will have a large population of individuals infected with streptomycin resistant strains.

Toxicity.

In the process of the development of streptomycin the organism Streptomyces griseus was found to produce histamine and histamine like substances. These substances were carried over into the finished product in the early preparations of the drug. These are now eliminated before it reaches the hands of the clinician.

The immediate toxicity of highly purified streptomycin on long continued administration is sufficiently low to justify the use of the drugs in the treatment of serious protracted infections such as most forms of tuberculosis. As streptomycin can produce potentially serious toxic reactions it is inadviseable to use the drug in the treatment of generally benign infections such as recently acquired pulmonary tuberculosis of minimal extent.

As with the other antibiotic drugs, streptomycin causes skin eruptions during the course of treatment. These are relatively common but are usually transient and not considered to be an indication for the discontinuance of therapy. Occasionally severe urticaria complicating streptomycin therapy

has been reported. Several reports have appeared in the literature of nurses having intimate contact with the drug showing epidermal sensitization to streptomycin.

The possibility of damage to the eighth cranial nerve has been referred to above.

Absorption and excretion.

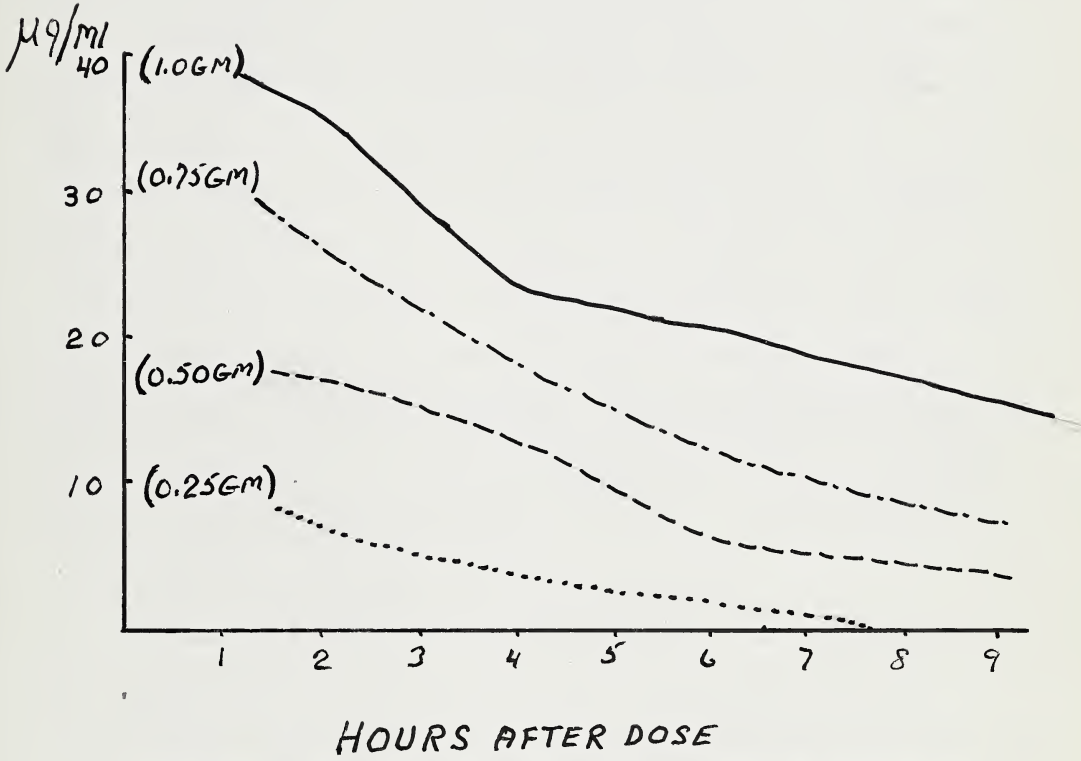
Following injection, streptomycin is readily absorbed and excreted. The usual method of administering the drug is by the intramuscular route and although intravenous administration has been used, it is no longer recommended, not only because of inconvenience to the patient but also because high blood concentrations are unnecessary. This is particularly true in the treatment of tuberculosis where prolonged high concentrations of the drug are not essential and in addition increase the possibilities of neurotoxic reactions.

As with penicillin it would appear from studies that have been made that the presence of an inflammatory reaction in the meninges increases the possibility of diffusion of streptomycin into the spinal fluid.

Oral administration of streptomycin is useless. It is not absorbed through the gut wall, and it does little more than develop rapidly resistant coliform organisms in the intestinal canal.

Streptomycin is absorbed more slowly following intramuscular injection than is penicillin and similarly is excreted at a slower rate. Blood concentrations may be maintained for approximately twelve hours following the injection of 0.5 gram streptomycin sulphate. (See Fig III).

FIGURE III



BLOOD SERUM CONCENTRATIONS OF STREPTOMYCIN FOLLOWING INTRAMUSCULAR ADMINISTRATION OF 0.25, 0.50, 0.75, AND 1.0 GRAM DOSES

Bacitracin

Bacitracin was first described by Johnson,Anker and Meleney in I945 (8) the result of a chance observation which, through true scientific curiosity,led to the isolation of this microbe destroying agent. While studying the bacterial flora of civilian wounds Johnson noted that,at times organisms could be isolated by plating debrided tissue on blood agar plates but that these organisms could not be found when the same material was inoculated into broth cultures. The broth cultures did,however show large numbers of a gram positive sporulating organism which proved to be Bacillus licheniformis.With the history of penicillin and tyrothricin before these scientists,it was natural that they should make a careful study of this phenomenon. One of the strains isolated from a patient by the name of Tracey appeared to have exceptionally strong antimicrobial activity and accordingly the antibiotic was given the name "Bacitracin".

It is well established that bacitracin is a polypeptide similar in this respect to polymyxin and subtilin. Although early results in animals with bacitracin indicated that it had a low degree of toxicityboth locally and systemically, it was soon demonstrated that this drug was nephrotoxic.This nephrotoxicity appears to be inherent in the drug itself and not to be due to impurities. As a matter of fact,it is firmly established that all of the polypeptide antibiotics including bacitracin,polymyxin,subtilin,and eumycin are nephrotoxic substances.The dose of these drugs must therefore be carefully controlled. In addition it is essential that the patients receiving the drug be carefully followed

for evidence of kidney damage.It is recommended that the urine of the patient be checked daily for evidences of albumen and that the blood be examined at least twice weekly for evidences of nitrogen retention,

Although bacitracin has certain toxic properties,it has some advantages over other antibiotics. Bacitracin rarely sensitizes patients treated with it and in this respect is superior to penicillin which when used locally causes a considerable number of allergic reactions.It appears that not only bacitracin but other polypeptide antibiotics are characterized by their negligible allergic potential.Another attribute of bacitracin is its marked synergistic action with other antibiotics particularly penicillin,as has been shown by Eagle and others.This synergistic action of bacitracin is made use of clinically particularly in the treatment of subacute bacterial endocarditis,wherein the infecting organisms are staphylococci or streptococci with a relatively high resistance to penicillin.

Bacitracin is primarily active against the gram positive bacteria but has little or no activity against the gram negative types except meningococci,gonococci and H,influenzae type B.

No bacitracinase has yet been found.

The mode of action of bacitracin against the gram positive organisms for which it is antagonistic is not known any more than is the mode of action of the other antibiotics.As with the others it is believed to act by interfering with certain enzyme systems that are essential for the development of these organisms.

In contrast to the action of aureomycin, chloramphenicol and terramycin, it will be observed that bacitracin has a considerably narrower spectrum.

Toxicity.

In man bacitracin has been shown to produce local pain and injury on intramuscular injection. At the site of injection petechiae of the skin and macular rashes have been observed. Some nausea and/or vomiting have also been reported and occasionally ringing of the ears. Of more importance however is the appearance of albuminuria and an increase in blood urea nitrogen in some patients.

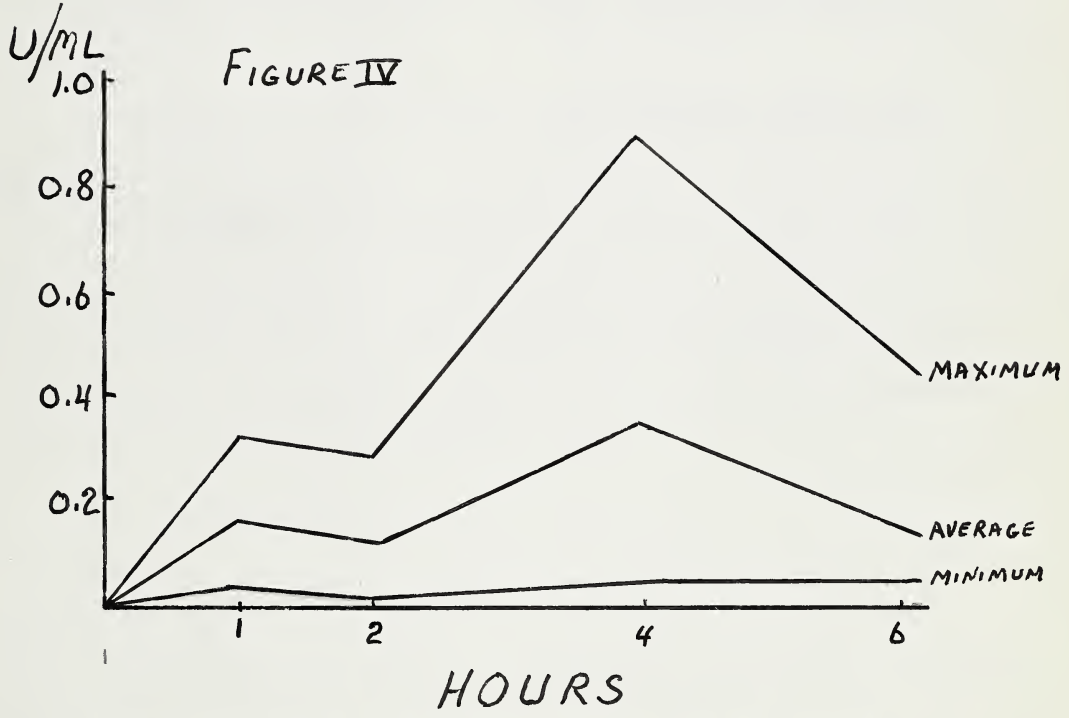
Absorption and excretion.

When 50,000 units or about one half the present maximum daily dose is administered intramuscularly in a single injection the peak concentration in blood develops by the fourth hour after which it gradually declines. Individuals vary greatly in their ability to retain bacitracin in their blood and variations from 0.03 to 0.8 units per ml after the injection of 50,000 units are found. (see fig.IV).

The oral administration of bacitracin does not ordinarily result in measureable blood concentrations, the greater portion of the total dose being recoverable in the faeces.

The parenteral administration of bacitracin as it is now available is a procedure that should be carried out only by the competent investigator, under conditions which will insure ready recognition of untoward effects. The usual dose should be about 50,000 units daily and should not exceed 100,000 units. Bacitracin has proven useful as a topical medicatment over

the past two years and its usefulness in surgical infections of a topical nature should not be minimized because of the possibility of its producing kidney damage following parenteral administration. It has proven to be a life saving drug in certain infections found resistant to other chemotherapeutic procedures.



BACITRACIN BLOOD CONCENTRATIONS FOLLOWING SINGLE INTRAMUSCULAR INJECTION OF 50,000 UNITS.

Aureomycin

Aureomycin was one of the first of the broad spectrum antibiotics. Its discovery was announced by Duggar in July I948 (9).He had obtained it as the result of a screening program and it is obtained from an organism Streptomyces aureofaciens.

This antimicrobial substance was found to be active not only against gram positive and gram negative bacteria but antagonistic to certain of the large viruses and Rickettsiae as well.

Aureomycin was found to be quite effective when given orally and at the present time the drug is used in most clinical conditions in this manner.Intravenous therapy is used in certain patients because of their inability to swallow or because of their moribund condition. In a number of these patients,because of inherent irritation of aureomycin or perhaps because of its acidity phlebitis occurs.

Up to the present time intramuscular therapy has not been utilized to any degree.In early clinical trials it was soon demonstrated that even with the concomitant use of a local anaesthetic the drug was extremely peinful when injected into the muscles.

The early studies of aureomycin indicated that it had an extremely low toxicity. As much as twenty four to thirty grams per day were utilized by Knight et al(IO)in the treatment of typhoid fever in Mexico without serious ill effects. In as much as the average dose is two grams per day this indicates a wide range between the therapeutic dose of this drug and

its toxic level.

The chemical structure of aureomycin is not known.

Recent evidence points to the possibility of a new vast field of usefulness for the drug in the field of nutrition. It appears that the addition of aureomycin in the form of the crystalline salt to standard diets increases the growth rate of chicken poults and swine by from twenty to forty per cent.

Fortunately organisms susceptible to the action of this drug do not develop resistance to it very rapidly. In this respect it differs markedly from streptomycin. Exceptions to this are strains of Proteus which do develop resistance to aureomycin with some degree of rapidity. Although S.typhosa is sensitive to aureomycin in vitro, it is of questionable value in the treatment of this disease in man. As with the other antibiotics it's mode of action is not known. It has a much wider spectrum of antimicrobial activity than either penicillin or streptomycin.

Aureomycin has been shown to be inactive by clinical trial in pulmonary tuberculosis. No dependent strains have as yet been demonstrated. No enzyme has been found that inactivates aureomycin as penicillinase does penicillin.

Toxicity.

Aureomycin can be used without fear of serious toxic effects. Untoward side reactions consist of nausea, vomiting, epigastric distress, heartburn, and diarrhoea which occurs more often in females than in males. Other side effects that have been reported following aureomycin are stomatitis, cheilosis, skin and mucous membrane eruptions and vaginitis. In addition

a Herxheimer type of reaction has occured consisting of chills fever and general malaise,nausea and vomiting,in cases of brucellosis treated with aureomycin. These conditions are not particularly serious and usually do not interfere with the treatment of the patient.

The diarrhoea observed in patients treated with aureomycin for a period of more than three days is probably not a toxic manifestation but rather as expression of the profound effect this drug has on the intestinal flora. In this respect it is similar to terramycin or, for that matter any combination of chemotherapeutic agents that are markedly active against both gram positive and gram negative aerobic and anaerobic bacteria of which the intestinal flora is composed. Often patients treated with these broad spectrum antibiotics for a period of three days or more show nothing but pure cultures of yeast in their stools. Not infrequently these stools become loose,lose their normal odor,and the patient complains of pruritis ani. The latter is probably due to the abnormal shift of the bacterial flora to the acid producing yeasts.

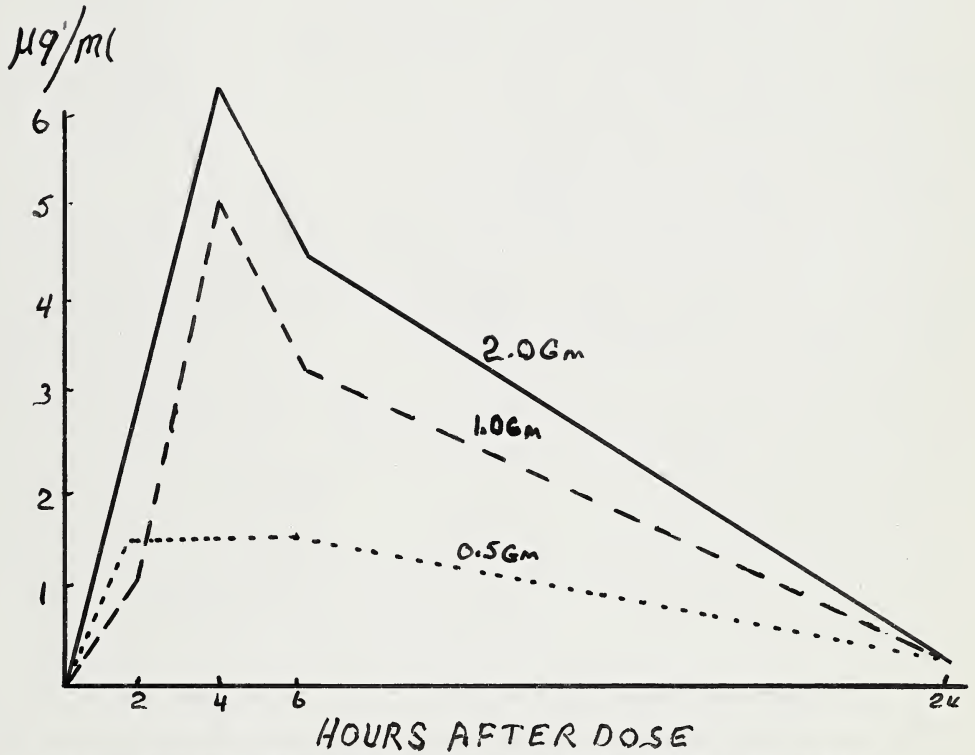
In comatose patients and those unable to swallow aureomycin intravenous has been used with considerable success. Unfortunately even with special diluents phlebitis occurs in approximately ten per cent of patients so treated.

Aureomycin like all drugs will sensitize some individuals. The evidence so far indicates that the incidence of patients who become sensitive to the drug is extremely low.

Absorption and excretion.

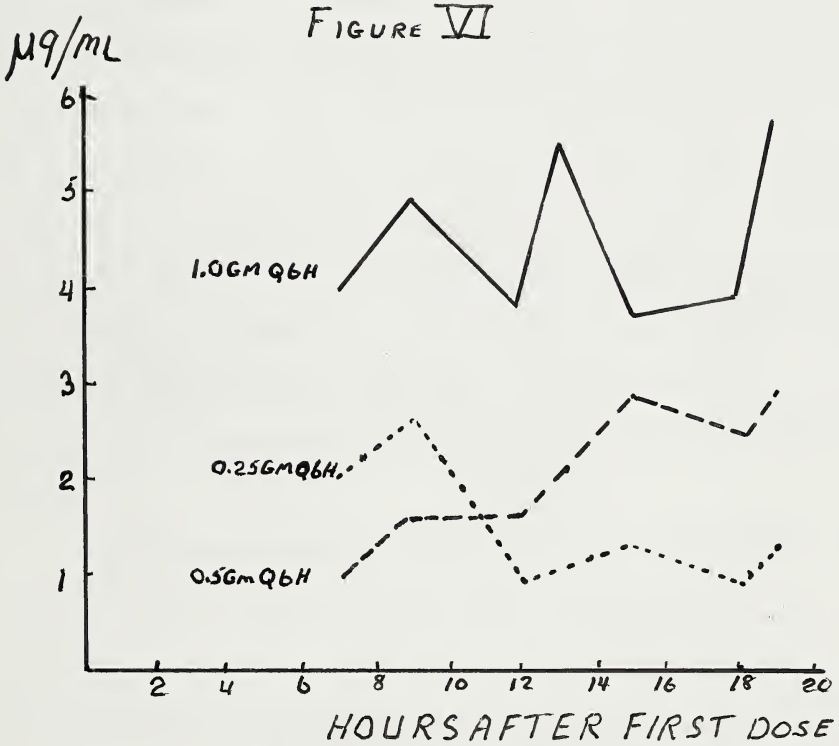
Aureomycin is readily absorbed from the intestinal canal when administered orally in capsule form. Following absoeption it diffuses into all body fluids and tissues. In single doses of from 0.75 to I.0 gram, therapeutic serum concentrations are obtained quite rapidly and these are maintained for relatively long periods of time. These serum concentrations are maintained at therapeutic levels rather constantly for from six to eight hours and complete disappearance from the circulation is not observed for from twenty four to thirty hours. When doses of 0.5 to I.0 gram are administered to patients every six to eight hours, the serum concentration of the drug is maintained at from two to four micrograms per ml, though the concentrations in some patients may exceed six or even eight micrograms per ml. From the circulation, aureomycin readily passes into the peritoneal fluid, spinal fluid, bile, urine, and milk. It passes into the fetal circulation through the placenta and has been found in the liver, kidney, lung and spleen. The studies of Herrell and Heilman (II) clearly show that aureomycin passes the blood brain barrier probably in therapeutic amounts and that the presence or absence of inflammation is not a factor controlling this diffusion as it is in the case of penicillin and streptomycin. (See Figures V and VI).

FIGURE V



BLOOD SERUM CONCENTRATIONS OF AUREOMYCIN FOLLOWING SINGLE ORAL DOSES.

FIGURE VI



BLOOD SERUM CONCENTRATIONS OF AUREOMYCIN FOLLOWING FOUR ORAL DOSES.

Chloramphenicol.

Burkholder of Yale is credited with the discovery of Streptomyces venezuela and Park Davis and company for preparingChloramphenicol from it in October I947.

It was soon found that chloramphenicol was a nitrobenzene compound previously unkown in nature. It contains both nitrogen and non-ionic chlorine. In contrast to penicillin, chloramphenicol was rapidly synthesized and commercially produced by a synthetic process. It is the first of the important antibiotics to be produced synthetically and on a practical basis. It is of value not only in many gram positive and gram negative infections but also in a number of diseases caused by Rickettsiae,spirochaetes,and viruses. In addition it is the only drug at present known that is specific in the treatment of typhoid fever.

The toxicity of chloramphenicol is relatively low. When administered by mouth it is well utilized,rapidly absorbed into the system and relatively high concentrations of the drug may be found in the body fluids. The side reactions such as nausea,vomiting and enteric symptoms are encountered perhaps less frequently than with the other broad spectrum antibiotics terramycin and aureomycin. Although,as may be anticipated with any drug,allergic phenomena are observed,fortunately these are relatively rare. It may be said at the present time that considerably fewer sensitizations occur with chloramphenicol than with,for instance penicillin and streptomycin.

Due to the rapid absorption of chloramphenicol from the gut and the rapid inactivation of that remaining little bactericidal effect is obtained on the bacterial flora. As a consequence yeasts do not gain preponderance as they do in patients treated with aureomycin and terramycin.

An interesting observation was made concerning the action of chloramphenicol on the cells from explants of embryonal brain tissue,fibroblasts and proliferating epithelial cells in tissue culture. Ten micrograms per ml which is in the range obtained with certain dose regimens retarded the growth of these tissues. In view of the large amounts of chloramphenicol that have been used clinically it does not appear that this effect on the growth of proliferating cells has any clinical significance.

Absorption and excretion.

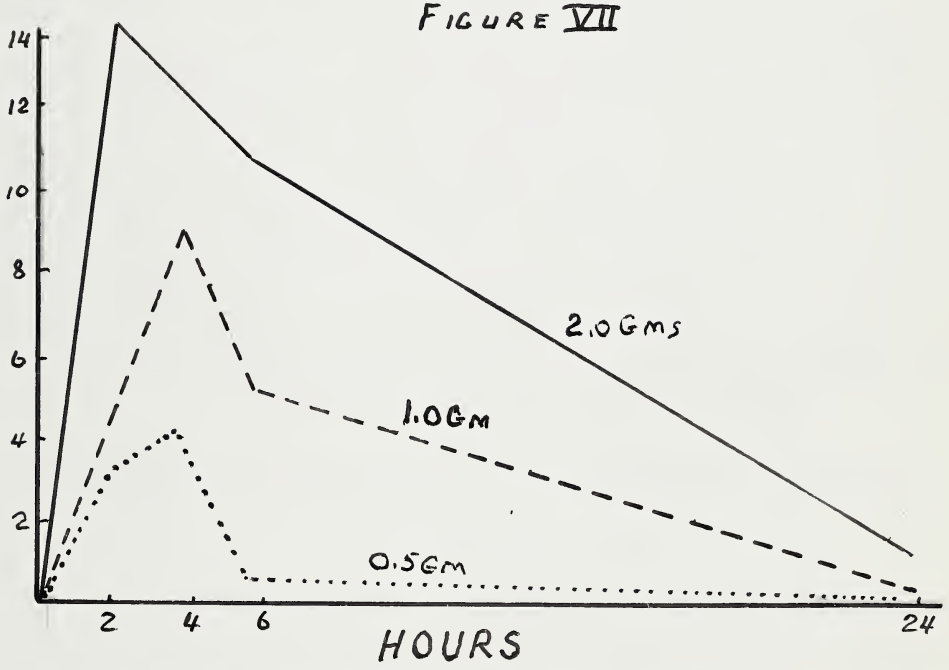
Chloramphenicol is very rapidly absorbed trom the intestinal tract in man. It is metabolized readily and excreted by way of the kidneys. Present evidence reveals that approximately ninety per cent of the administered dose may be accounted for in the urine in a period of twenty four hours.

The average blood concentrations of active chloramphenicol with single doses are shown in figure VII.

It will be noted that two hours after the dose had been given,by comparison with similar data on aureomycin and terramycin ,higher concentrations were obtained than with the other two drugs. The concentration of chloramphenicol in the blood drops off rapidly so that by the sixth hour its concentration issomewhat lower than obtained at this time by either aureomycin or terramycin.The more rapid disappearance of

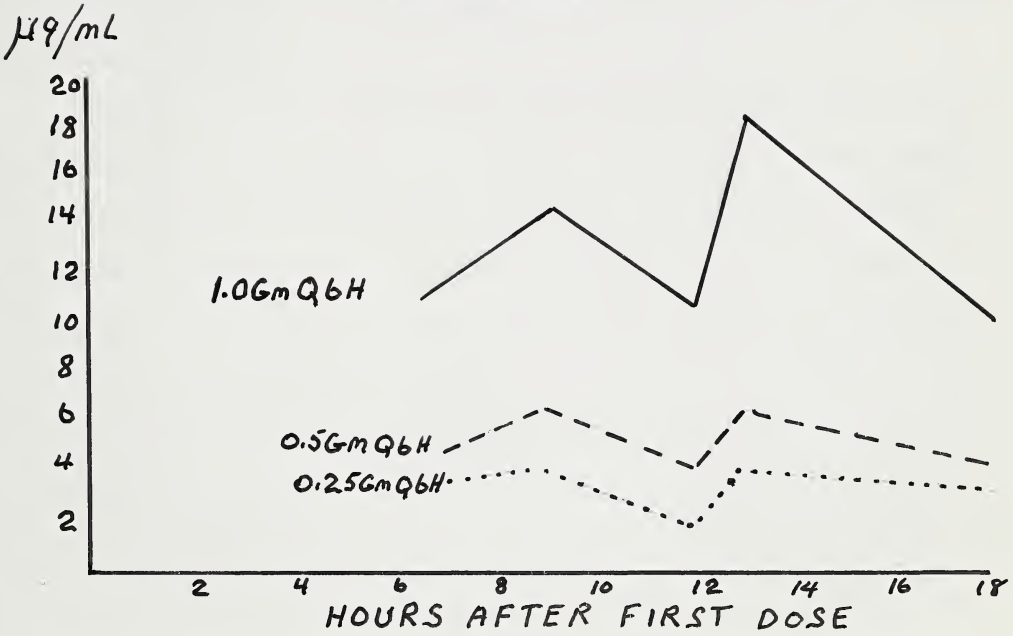
chloramphenicol may be explained by the fact that it is more rapidly metabolized into the inactive nitro compounds.It is apparent from a study of the figure that all subjects used maintained significant blood concentrations of chloramphenicol for a period of at least six to eight hours. In the therapeutic use of the drug,proper dosages at these intervals have been shown to be clinically effective.

FIGURE VII



BLOOD SERUM CONCENTRATIONS OF CHLORAMPHENICOL FOLLOWING SINGLE ORAL DOSES.

FIGURE VIII



BLOOD SERUM CONCENTRATION OF CHLORAMPHENICOL FOLLOWING DIVIDED ORAL DOSES.

In comparison with terramycin,the amount of active chloramphenicol excreted in the urine is considerably lower. This lower excretion,however,cannot be taken as an indication of lack of activity in urinary tract infections.Indeed it has been shown to have an extremely high chemotherapeutic activity in these diseases.

A wide clinical experience has been obtained with chloramphenicol in both acute and chronic conditions. These experiments indicate that the great majority of patients under treatment respond well to a dose of approximately fifty mg. per kg.of body weight per day. This dose is given in divided doses for several days or until the signs and symptoms of the disease under treatment have disappeared.When the patient becomes afebrilethe dose may be reduced to twenty five mg.per kg. of body weight per day. An analysis of the data available on dosages used for adults has established that a total dose of chloramphenicol of ten to fifteen grams is adequate for most conditions but for chronic diseases fifteen grams or more are required. As with terramycin and aureomycin it is possible to omit the night doses of this drug.The interval between doses should not exceed eight hours.In infants and children doses of chloramphenicol should probably be calculated on the basis of one hundred mg.per kg.body weight per day.

For some time there has been a reluctance on the part of practitioners to use this very valuable drug for fear of causing a blood dyscrasia.This fear now appears to be without real foundation and if the drug is used in the manner suggested

above,no ill effects are to be anticipated.

Terramycin.

The isolation of terramycin from culture filtrates of a new actinomycete,Streptomyces rimosus,was reported in Science during January I950.(I2)

Isolation of this chemotherapeutic agent was the result of a carefully designed screening program. The element of chance was not entirely eliminated,but certainly it was reduced to a minimum by the team of scientists that were responsible for its discovery.Although chance was involved in the origional isolation of the proper colony by Routien, there was no element of luck involved in the isolation characterization,and final crystallization of the drug itself.

Terramycin is an amphoteric compound and forms both the crystalline hydrochloride and sodium salt. It may be considered to be a very stable drug as the hydrochloride does not lose significant potency even in aqueous solution at room temperature over relatively long periods of time.It is the third of the so-called broad spectrum antibiotics and is active against a variety of microorganisms,including both gram positive and gram negative types. In addition the early experimental work on this drug showed it to be active against Rickettsiae and certain of the viruses both in vitro and in vivo. The drug is well tolerated when given orally and is absorbed from the gastro-intestinal tract in sufficient amounts to give effective concentrations in the blood and urine. Terramycin,like aureomycin,increases the growth rate of

chickens, poults, and swine when added in small amounts to their diet.

At the present time, terramycin is used in the form of the hydrochloride which is highly water soluble. It is administered usually by mouth in capsule form. The drug has a relatively low toxicity and may be taken in large doses without fear of serious side effects. Nausea and vomiting have been reported with relatively high doses but these usually do not preclude continued administration of the drug. The rather profound effect of terramycin on the intestinal flora, with elimination of many of the so-called normal inhabitants of the intestinal tract, usually results in the development of soft stools which may constitute diarrhoea. This side effect is not serious and ceases promptly on withdrawal of the drug.

The three antibiotics described as the broad spectrum antibiotics, chloramphenicol, aureomycin, and terramycin have been made available to the general practitioner since October 1947. Although these three antibiotics have much in common they are different compounds. It is obvious that chloramphenicol which has been chemically identified and synthesized is markedly different from the other two and although terramycin and aureomycin have many characteristics in common they are definite chemical entities. However the fact that all three of these antibiotics were derived from actinomycetes and streptomyces species, that all three are characterized by wide antimicrobial activity against gram positive and gram negative bacteria as well as certain Rickettsiae and large viruses

that all three have a low degree of toxicity in both man and animals, that all three at proper dosage levels are equally effective in certain diseases of man, and that in the case of terramycin and aureomycin they are almost the same yellow colour, has led some individuals to consider them identical. Differences in these three drugs may be readily demonstrated in addition to the obvious differences in the chemical structure of chloramphenicol from aureomycin and terramycin. Further, it is well established that chloramphenicol is an active agent in the treatment of typhoid fever while the other two are not. Terramycin is more active in vitro against some of the clostridia than the other two, whereas aureomycin in its turn has advantages over terramycin and chloramphenicol in the treatment of certain staphylococcal infections.

The wide antibacterial spectrum presented in the original report on terramycin has been confirmed by Hobby et al (13), Welsh et al (14), and others. Antimicrobial activity was shown against a variety of aerobic and anaerobic gram positive and gram negative bacteria and certain Rickettsiae. The drug is quite active against the clostridia and saprophytic bacteria but it is inactive against yeast like organisms and Proteus vulgaris. The drug is, like aureomycin largely bacteriostatic rather than bactericidal.

It should be emphasized that there is a variation in the sensitivity of strains of the same species of bacteria in their response to any antibiotic. It is thus highly desirable to determine the sensitivity of the infecting organisms in each

case in order to evaluate properly the therapeutic usefullness of the antibiotics.

Pharmacology.

Terramycin has proven to be a drug of extremely low toxicity.This low toxicity of terramycin by mouth is evident from the fact that doses of at least fifteen grams per day over a period of ten to fifteen days have been given without evidences of serious toxic effects.

Terramycin has been administerdd largely in capsule form by the oral route.Patients treated by this mode of administration rarely show untoward reactions. There is evidence that terramycin causes gastrointestinal irritation in some patients. This is manifested by nausea and on occasion by vomiting and diarrhoea,These evidences of toxicity are usually minor in character.Other reactions reported include proctitis vaginitis,and pruritis,usually of mild degree.

Terramycin is now available for intravenous administration. This preparation should not be used except in these patients who for one reason or other are unable to take the drug by mouth. The relatively high alkalinity of the solution may cause phlebitis.

The close relationship between terramycin and aureomycin is readily demonstrable in the absorption and excretion of these drugs following oral administration. Both drugs are well absorbed from the gastro-intestinal tract and are easily demonstrated in the blood and urine.In addition appreciable amounts are excreted in the faeces and thereby alter the intestinal flora.As with aureomycin complete disappearance of terra

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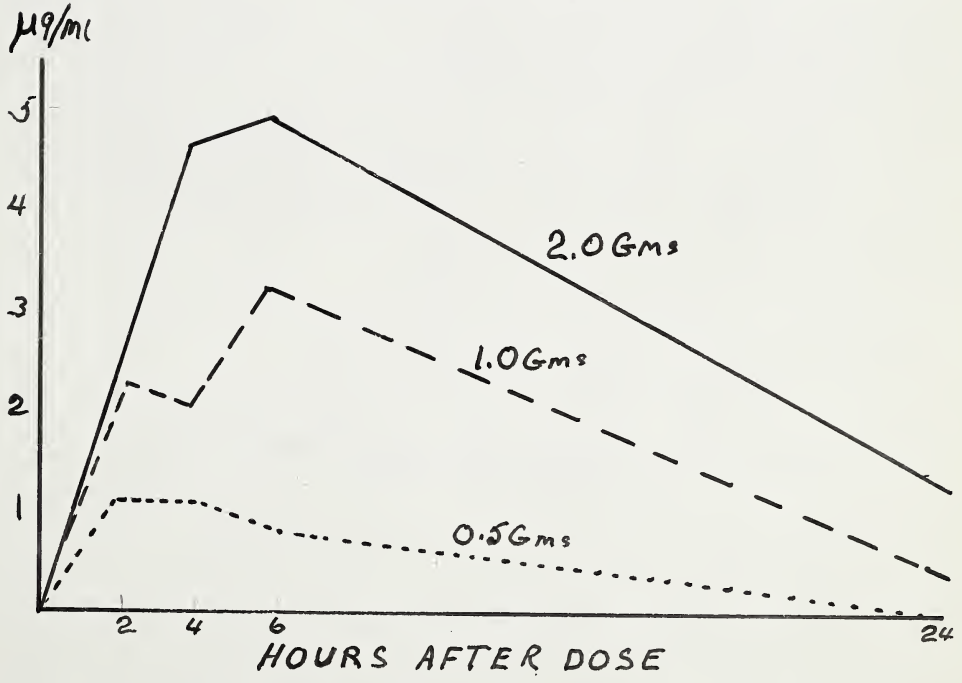
terramycin from the blood serum may occur as late as the twenty fourth hour after single doses. There is,however,a definite diminution in the amount of terramycin in the blood six to eight hours after single doses of 0.5 to 2.0 grams. Terramycin does not readily traverse the blood brain barrier as aureomycin does.In cases where the meninges are inflammed terramycin has been demonstrated in the spinal fluid. It does diffuse readily into pleural fluid,bile,and through the placenta into the foetal circulation.

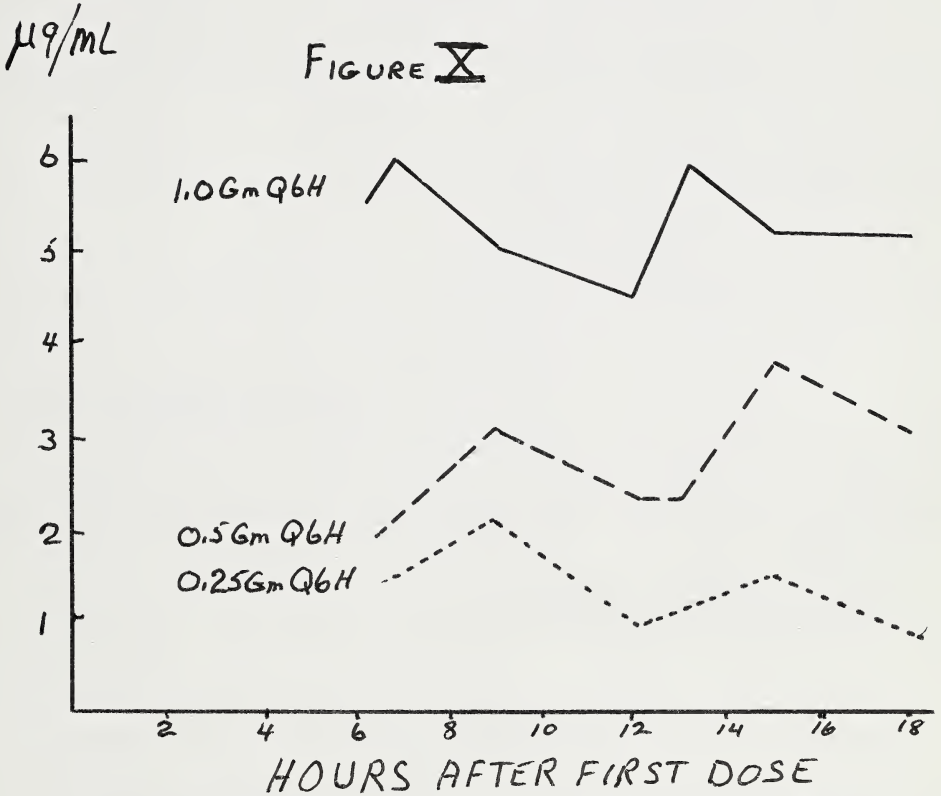
Blood concentrations of terramycin can be maintained at a relatively high level by oral administration every six hours.

FIGURE IX



Blood serum concentrations of terramycin following single oral doses.

FIGURE X



Blood serum concentration of terramycin following four oral doses.

Tests for Organismal Sensitivity.

It should always be remembered that the only absolute criterion of microbial response to antibiotics in vivo is the clinical response of the patient when adequate dosage of the antibiotic is administered. All other (i.e. in vitro)measurements carried out prior to therapy are artificial and the quantitative data yielded might only be considered to point out an approximate range of antibiotic concentration in which there is definite interference with growth and/or reproduction of incriminated pathogenic strains.The methods of testing for organismal sensitivity presently in use are known as the tube dilution method and the disc method.

The tube dilution method.

A fresh tube of antibiotic stock solution containing 1000 units or micrograms per ml. is removed from storage as needed. By diluting one part of stock solution with four parts of broth diluent one obtains working solutions of 200 units or micrograms per ml.

Pipette 0.5 ml. of the dilution broth into the second through the tenth of a series of sterile test tubes prepared for each sensitivity test. Into the first and second tubes then pipette 0.5 ml. of the 200 unit or microgram working solution. Mix the contents of the second tube well and transfer 0.5 ml. from the second to the third tube and from the third to the fourth etc.,mixing well before each transfer. Discard 0.5 ml. from the ninth tube. The tenth tube receives no antibiotic and serves as a control. To all tubes now add 0.5 ml. of a 1/10,000 dilution (in broth) of an eighteen to twenty-

four hour culture of the test organism grown at 37 degrees C. The final volume in each tube prior to incubation is I.Oml. and the antibiotic concentration range covered in the series is from I00 to 0.39 units or micrograms per ml. in two fold steps. To extend the range of concentration covered dilutions may be carried byond tube No.9 in the series.

The series is usually incubated overnight at 37 degrees C and examined macroscopically for evidence of growth. The lowest antibiotic concentration in each case which prevents growth (as manifested by turbidity) is taken as the minimal inhibiting concentration (M.I.C.),sensitivity or antibiotic susceptibility value. See Table I and Plate I.

The disc method.

The tube dilution method of determining orgainismal sensitivity is time consuming and if a large number of sensitivities are to be done a very large staff and a very large amount of apparatus would be required. As a consequence,of recent times, the disc method has been brought into use more and more simply because many sensitivities can be determined on one blood agar plate and require only a few moments to place in position.

The disadvantages of the disc method are related to the narrowrange of dilutions that can be obtained when multiple sensitivities are determined on one plate.

The disc method is particularly useful when the clinician has found that his patient has not responded to the antibiotic which he had believed would be effective.A rapid answer can be obtained by submitting a specimen from the infected area

to the laboratory. The physician must rely on the laboratory for the determination of the sensitivity of microorganisms in cases where prompt response is not obtained with the usual antibiotic therapy.

The specimen can, if the matter is urgent, be used as the inoculum.

Closely similar results were obtained when the disc method was run in parallel with the serial dilution tube method and it is reported that the disc technique is as accurate as the more complicated tube dilution method for most purposes.

In vivo sensitivities cannot be accurately gauged from in vitro levels thus mathematical expressions, if used might be misleading.

The results obtained with discs are of a qualitative rather than a quantitative nature. There are many variables that enter into a test of this type such as: the size of the inoculum, type of growth of the organism, thickness of the medium, incubation conditions, diffusion rate of the antibiotic, rate of deterioration of the antibiotic during incubation. All of these preclude its quantitative nature. Sensitivities are determined by the presence of and not entirely by the diameter of the zone of inhibition around the disc.

The information obtained by the use of sensitivity discs is not only an important aid in determining which antibiotic may be effective against a specific infection but also which antibiotics are ineffective. See table II and plates II and III.

Table I.

Tube Dilution Method

Tube Set-up.

Tube No.	Diluent (medium) added	Antibiotic added	Diluted culture added	Final antibiotic concentration units or mcgs.
I	none	0.5 ml working soln	0.5ml	I00
2	0.5ml	0.5 ml working soln	0.5ml	50
3	0.5ml	0.5 ml from #2	0.5ml	25
4	0.5ml	0.5 ml from #3	0.5ml	I2.5
5	0.5ml	0.5 ml from #4	0.5ml	6.25
6	0.5ml	0.5 ml from #5	0.5ml	3.I25
7	0.5ml	0.5 ml from #6	0.5ml	I.56
8	0.5ml	0.5 ml from #7	0.5ml	0.78
9	0.5ml	0.5 ml from #8*	0.5ml	0.39
I0	0.5ml	none	0.5ml	zero

* discard 0.5 ml from tube No.9

Table II.

The Disc Method.

Concentrations of antibiotics.

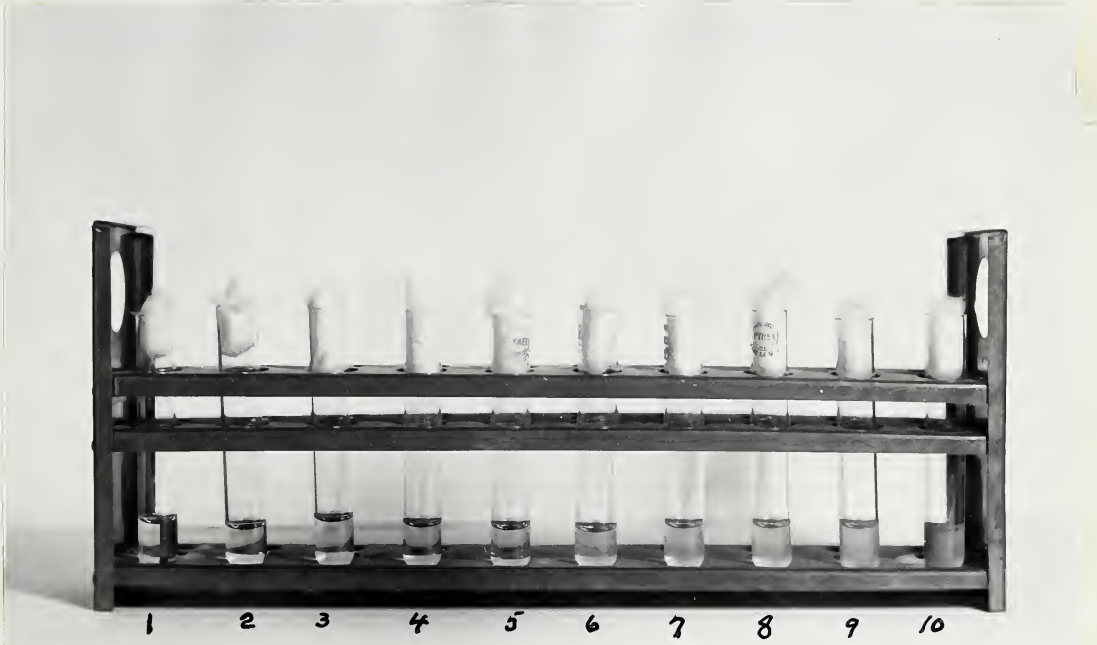
The concentration of antibiotic on discs as prepared at the Provincial Laboratory up to 1 June 1954.

Penicillin	1 unit
Aureomycin	35 micrograms
Chloromycetin	8, 30, 60, micrograms
Terramycin	10, 30, 60, micrograms
Streptomycin	4 micrograms
Erythromycin	1 microgram

Early in 1954 it was announced that the antibiotics Aureomycin and Terramycin were found to be the related chemical compounds chlortetracycline and oxytetracycline. About the same time a new preparation Achromycin (which is the basic active principle, tetracycline) was placed on the market.

The Provincial Laboratory therefore has discontinued the routine use of Aureomycin and Terramycin discs and has substituted Achromycin.

Plate I

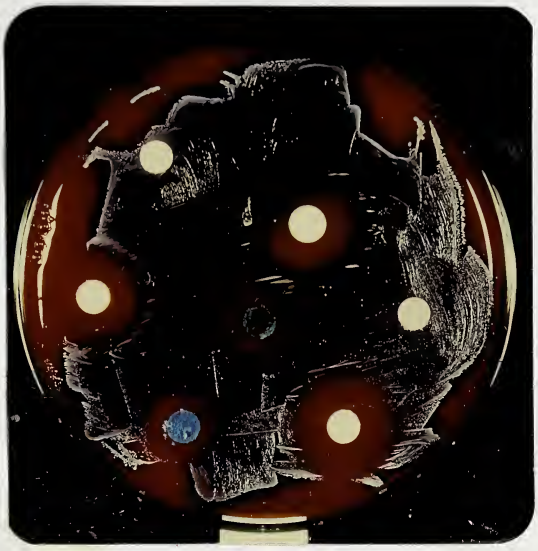


Antibiotic — Penicillin

Test organism — Staphylococcus aureus

Minimal Inhibitating Concentration — 6.25 units/ml (5th tube)

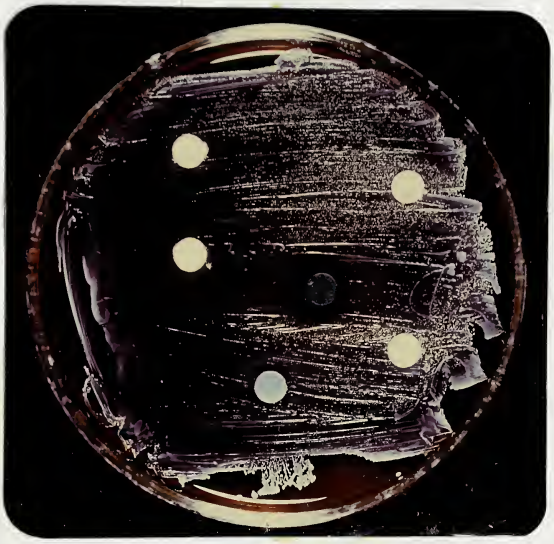
Plate II



This picture shows a culture of S.aureus inhibited by discs of aureomycin,chloromycetin,terramycin,erythromycin, and furadantin. It is resistant(i.e.there is no clear zone on inhibition surrounding the disc) to penicillin and to streptomycin.

The discs are made by dropping onto pieces of absorbent paper solutions of antibiotics as set out in Table II. The discs are stored in the refrigerator to prevent deterioration.

Plate III



This picture shows a culture of a different strain of S.aureus from that illustrated in Plate II. This Staphylococcus is resistant to penicillin,aureomycin,chloromycetin, terramycin,and streptomycin. It is however sensitive to erythromycin though the area of inhibition is not as clearly defined as those in Plate II.This demonstrates the need of sensitivity testing in order that an effective antibiotic may be selected. It illustrates too,the use of erythromycin a relatively new antibiotic,in infections due to gram positive cocci which are resistant to the other antibiotics.

Present conceptions as to the mode of antibiotic action.

Many years ago Ehrlich (15) recognized that the efficacy of a chemotherapeutic agent depends largely on the differences in its toxicity for the parasite and the host. At that time it was characteristic of all the compounds used in chemotherapy-the metals,phenols and dyes,for instance-that though they had some selective action,mainly on the gram positive bacteria,their power to kill microorganisms varied with their concentration,and doses which were lethal to the invading organism were usually toxic to the patient.

Penicillin

Penicillin excels in its lack of toxicity. Its most important effects on bacteria have been thought to be a progressive disturbance of the passage of glutamic acid into the organism. This is believed to be brought about by its action on the cell wall disrupting the metabolism of ribonucleic acid thus preventing the normal assimilation of glutamic acid which is required by the cell for protein synthesis.

The sensitivity of the gram positive organisms and the immunity of the gram negative ones is explained by the fact that in the latter glutamic acid is synthesised inside the cell and does not have to be taken in from outside.

It may be emphasized that this action of penicillin is on the growing cell and it is without any effect on non - growing organisms. (17)This has been proven by an experiment which indicates utilization of added penicillin by growing cells at 0 and 18 hours while controls of killed organisms and plain broth show no decrease in the amount of the drug.

(see table IV)

Organisms which become penicillin resistant assume some of the properties of gram negative organisms, that is they acquire the ability to synthesise glutamic acid and need not then assimilate it from their environment so attaining an independence against which penicillin is powerless to act. These differences in the amino acid metabolism of bacterium and host are the nutritional basis on which penicillin acts.

Streptomycin.

Streptomycin differs from penicillin in that it affects not only the growing organisms but those in the resting phase as well. Its bactericidal effect increases with higher concentrations. The action of streptomycin seems to be more complex and is less well understood. It is known, however, that it interferes with the oxidation of threonine and serine in gram negative organisms as well as with the oxidation of fumarates and pyruvates (18) (19) or even in a later part of the catabolic process, the citric acid cycle (20). (see fig.XI).

Resistance to streptomycin develops rapidly in one to five days. Bacterial mutants actually incorporate the streptomycin into their metabolic cycle (21).

The broad spectrum antibiotics.

The so called broad spectrum antibiotics such as aureomycin, terramycin, and chloromycetin interfere with the synthesis of adenosine triphosphate which is one of the energy producing materials used in the synthesis of more complex cellular components. Resistance in the case of the broad spectrum antibiotics develops slowly in a step-like fashion. It may develop two to four times, but strongly resistant, fast, or

dependent strains are not common.

Cross resistance to antibiotics.

Many workers have investigated in parallel experiments the resistance of individual bacteria to all the currently used antibiotics. This inter association of resistance is particularly important in clinical treatment, and especially in relation to the choice of an alternative antibiotic when an organism has become resistant under treatment.

It was found that resistance to penicillin was entirely unrelated to resistance to the others and that the resistance to aureomycin, terramycin, and chloromycetin was closely related, particularly in all gram negative species but less easily acquired and less evidently related in gram positive cocci and bacilli.

Resistance between aureomycin and terramycin was found to be closely linked which is now understandable as their very similar chemical structure is known. It is apparent therefore that the substitution of terramycin for aureomycin or vice versa is not likely to be helpful in therapy. The association of resistance to these two antibiotics with chloromycetin was not so pronounced. Most workers found little relationship between streptomycin resistance and resistance to the three broad spectrum antibiotics.

Synergy and antagonism between antibiotics

With the growing appreciation of theincrease of bacterial resistance to one or other of the usual antibiotics and of the unfortunate therapeutic repercussions that have developed in certain quarters, there is a tendency to use anti-

biotics

in combination or even in the shot gun principle that if there are enough antibiotics in a mixture one is bound to hit the target.

Laboratory experiments quite early showed that antibiotics could not be used as simple mixtures and that such mixtures might be antagonistic as in the case of chloromycetin and penicillin. Interference was also observed of aureomycin and terramycin with penicillin in experiments with mice infected with Streptococci or Klebsiellae.

The explanation of this phenomenon is of course the fact that the broad spectrum antibiotics are mainly bacteriostatic and tend to check bacterial growth thus removing the condition necessary for bactericidal action particularly of penicillin and to some extent of streptomycin, namely active multiplication.

Although the recorded incidence of antagonistic effects makes one hesitate to use a combination of drugs empirically, there is the occasional demonstration of a distinct synergistic action that may give rise to an important therapeutic effect. Thus the simultaneous administration of penicillin and streptomycin has cured a number of cases of bacterial endocarditis due to enterococci where the individual drugs have failed.

From experimental work such as mentioned above antibiotics have been divided into two groups in relation to their synergistic activities.

Group I includes penicillin, streptomycin, bacitracin, and neomycin which were found to be synergistic and never antagonistic.

Group II comprises aureomycin, chloromycetin, and terramycin which were neither synergistic nor antagonistic but may show simple additive effects on combination.

Certain principles may be established from the knowledge gained so far to aid the physician in his choice of antibiotic combinations. Group I antibiotics are never antagonistic but are often synergistic provided that the test organism is somewhat susceptible to at least a high concentration of each drug used. The effect of combinations of Group I and Group II drugs depends on the sensitivity of the organisms to Group I. Thus (a) if highly sensitive to Group I the addition of Group II drugs is usually antagonistic, (b) if relatively resistant to Group I and somewhat susceptible to Group II agents then the combination of I and II occasionally yields synergistic effects. Antagonism is a unilateral action and drugs of Group I have never interfered with drugs of Group II.

In all this work on synergism and antagonism the recorded effects have been in terms of bactericidal action. This is probably of important clinical application when the rapid killing of organisms is essential as in diseases such as endocarditis, meningitis, and acute septicaemias. No information regarding synergism and antagonism in respect to bacteriostatic action is yet available. This is probably of considerable importance, particularly in the clinical application of antibiotic combinations in diseases where the active cellular and humoral resistance of the patient can be brought to bear.

Table IV

A young (8 hour)broth culture of S.aureus was divided into two parts.One part was heated at 70 degrees C to kill the organisms and then colled to room temperature. The other part contained young growing organisms.

One ml of penicillin(10 units/ml) was added to two tubes each containing 4 ml of killed organisms,two tubes containing 4 ml of growing organisms and to similar quantities of plain broth to act as controls.

These mixtures were arranged in two lots,one of each being incubated for 8 hours and the others for 12 hours.

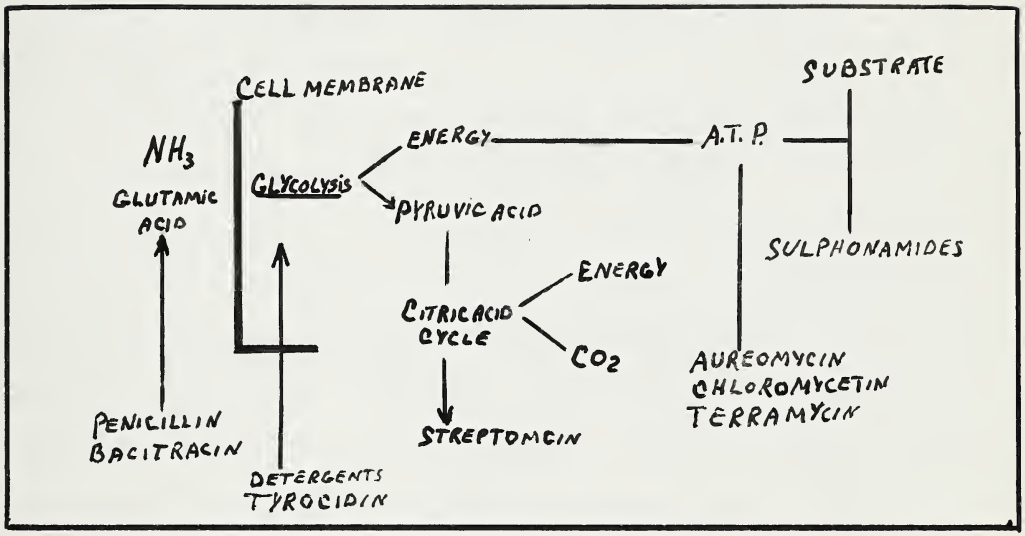
On removal from the incubator the tubes containing organisms were centrifuged and the supernatent set up along with the broth controls in a series of tubes to which a test organism was added.These were incubated overnight.The dilutions of the control were calculated to be:

Tube No.	Units penicillin
1	0.66
2	0.165
3	0.0825
4	0.0412
5	0.0206
6	0.0103

It was found that the minimal inhibiting concentration in the control and killed organism series occured in the 4th tube but in the growing organism tests the minimal inhibiting concentration was in the 3rd. As a similar quantity of penicillin was origionally added this indicated that some of the penicillin had been taken up by the growing cells while in contact with them.

	Tube showing minimal inhibiting concentration		
Hours of contact before addition of test organisms	Plain broth plus penicillin	Killed organism plus penicillin	Growing organism plus penicillin
8	4	4	3
12	4	4	3

FIGURE XI



Site of Antibacterial action.

The Relationship between Sensitivity Tests and the Clinical Use of Antibiotics

In order to determine the relationship between sensitivity tests as performed in the Provincial Laboratory (by the disc method) and the clinical use of antibiotics, the case records of patients in the University Hospital whose physician had requested such tests durin ghe year 1953 were examined.

Of the 832 case histories drawn from the record room, 334 were found suitable for study and extracts were made from them. The balance were rejected either because the patient's stay in hospital was too short to determine his progress, or the case was that of advanced carcinoma, cerebral haemorrhage, or other disease of a terminal nature. In some instances the patient did not receive any antibiotic and in others one of the sulpha group alone was administered.

It may be categorically stated that with few exceptions all cases in which a rapid cure of the patient's infection was achieved, there was revealed an organism sensitive to an antibiotic suggested by the laboratory and that this antibiotic was given in adequate dosage.

An example of good therapeutics is seen in case M3068. This patient was admitted suffering from lobar pneumonia. This diagnosis was confirmed clinically and by chest x-ray. The attending physician (a) started the patient on crystalline penicillin followed by S.R.penicillin immediately upon admission and (b) sent a specimen of sputum to the laboratory to confirm his impression that the causitive organism was D. pneumoniae.

The laboratory confirmed that the invading microorganism was D.pneumoniae and that it was sensitive to penicillin, aureomycin, chloromycetin and terramycin.

Should the organism have been, for example, a penicillin-resistant staphylococcus the physician would have been able to change his therapy in the minimum length of time. In this instance a rapid cure was obtained. (see chart I).

A second case M2931 was admitted, suffering from septicaemia originating in an infected finger and it further illustrated the efficacy of the correct antibiotic. (see chart II).

In a number of instances the attending physician did not give an effective antibiotic at the time the patient was first admitted (75 in this series). This was due usually to the fact that a report identifying the invading microorganism and suggesting an effective antibiotic either (a) had not been asked for or (b) was asked for but not received until after treatment had been started.

The result of this delay meant a longer period of treatment in hospital prior to the patient regaining his health. (see table II).

At times this delay was unavoidable as is illustrated by case M2420. This elderly female suffered from an intra-abdominal infection and it was not until a laparotomy was performed could the surgeon obtain a specimen for submission to the laboratory. Once this was obtained and the correct antibiotic given the patient quickly recovered. (see chart III). In this case it should be emphasized that no free pus was evacuated and that her recovery was due entirely to the action of the antibiotic on the invading organism.

CHART I

DATE

DAY IN HOSPITAL

DAY OF DIS.

Hour M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E.

TEMPERATURE

106° 105° 104° 103° 102° 101° 100° 99° Nor. 98° 97° 96° 95°

M3068 D. PNEUMONIAE SENSITIVE TO PENICILLIN
AUREOMYCIN
CHLOROMYCETIN
TERRAMYCIN

PNEUMONIA LEFT LOWER LOBE

S.R. PENICILLIN 400,000 @ 10

PENICILLIN CRYSTALLINE 300,000 UNITS

PULSE

160 150 140 130 120 110 100 90 80 70 60

RESPIRATION

50 40 30 20 10

BLOOD PRESSURE

FLUID INTAKE

URINE

DEFECATION

WEIGHT

CHART II

DATE

DAY IN HOSPITAL

DAY OF DIS.

Hour M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E.

TEMPERATURE

106° 105° 104° 103° 102° 101° 100° 99° Nor. 98° 97° 96° 95°

M 2931

STAPHYLOCOCCUS AUREUS
STREPTOCOCCUS HAEMOLYTICUS
SENSITIVE TO - PENICILLIN
AUREOMYCIN
CHLOROMYCETIN
TERRAMYCIN

SEPTICAEMIA

TOTAL 6 MILLION UNITS PENICILLIN

CRYSTALLINE PENICILLIN 200,000 Q 3H

S.R. PEN 400,000 T.I.D

S.R PENICILLIN 400,000 B.I.D

PULSE

160 150 140 130 120 110 100 90 80 70 60

RESPIRATION

50 40 30 20 10

BLOOD PRESSURE

FLUID INTAKE

URINE

DEFECATION

WEIGHT

Table III

Number of days under treatment

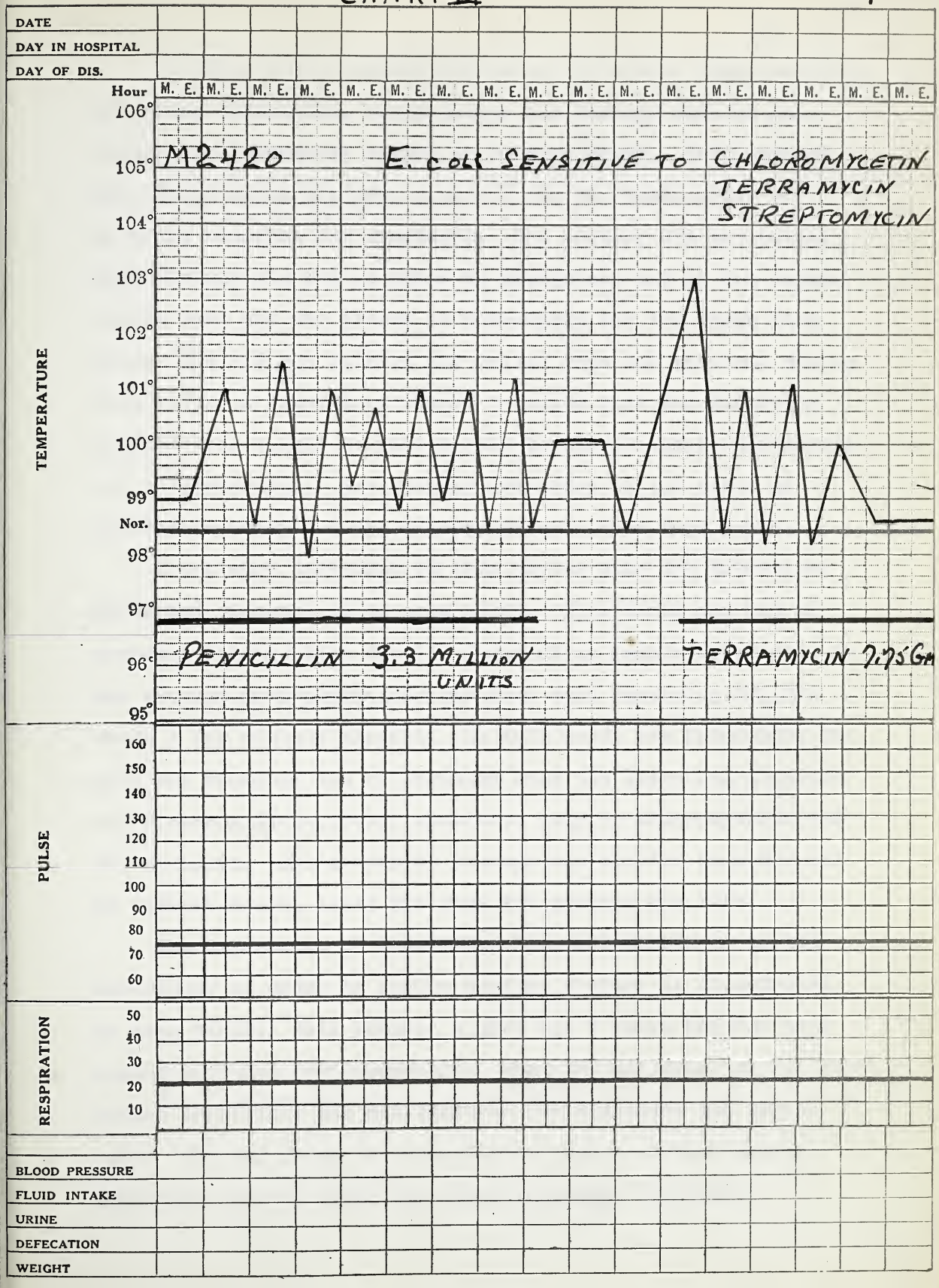
	Cystitis	Chronic Infect.	Post op. Infections	Superficial Infections
Group A	7	6	6	7
Group B	8	12	13	14
Group C	14	18	19	25

Group A are those cases in which the correct antibiotic was used from the first.

Group B are those cases in which two antibiotics were administered one of which was specific for the invading organism.

Group C are those cases in which more than two antibiotics were used - one of which or more than one were specific for the invading organisms.

CHART III


DATE
DAY IN HOSPITAL
DAY OF DIS.
Hour
M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E. M. E.
TEMPERATURE
106°
105°
104°
103°
102°
101°
100°
99°
Nor.
98°
97°
96°
95°
M2420
E. COLI SENSITIVE TO CHLOROMYCETIN
TERRAMYCIN
STREPTOMYCIN
PENICILLIN 3.3 MILLION UNITS
TERRAMYCIN 1.75 GM
PULSE
160
150
140
130
120
110
100
90
80
70
60
RESPIRATION
50
40
30
20
10
BLOOD PRESSURE
FLUID INTAKE
URINE
DEFECATION
WEIGHT

Usually, the attending physician, if he was administering the wrong antibiotic, would change his therapy when he received the laboratory report as in case M4025. This patient age 32 was suffering from pyelitis for two weeks prior to entering hospital for treatment. The history did not reveal what therapy she was on prior to admission, and it was unfortunate that she was placed on aureomycin for the first five days. She did not receive any relief from her symptoms during that time. A laboratory report revealed that the infecting organism was E.coli sensitive only to Streptomycin, Terramycin and Chloromycetin. She quickly responded to I.5 grams of Terramycin a day and was discharged well four days later.

There were instances in this review when the laboratory prevented disaster as in case M3580. This young man had an operation on his knee which unfortunately became infected. He was treated with penicillin for four days receiving 600,000 units a day without benefit. At this point the laboratory report was received and it revealed that the infecting organism was S.aureus (haemolytic) sensitive only to Chloromycetin and Erythromycin. Chloromycetin therapy was started that day and it without doubt, saved the knee and perhaps his life.

The importance of submitting frequent specimens to the laboratory in order to ensure correct therapy is illustrated by case M5151. This patient a girl age 7 years had for many months suffered from eneuresis. When the situation at her home became impossible she was admitted for diagnosis and treatment. She was found to be suffering from a chronic pyelonephritis and the first two urine specimens contained

E.coli sensitive to terramycin,chloromycetin,and streptomycin. Following four days of terramycin she had improved but was not cured.The next two specimens of urine grew S.aureus (haemolytic) which was probably the basic cause of her trouble. This organism was sensitive only to chloromycetin. The E.coli reported present in the two first specimens had been eliminated as it did not appear again. Specific therapy with chloromycetin cured her completely in four days.

In some instances two antibiotics were prescribed before the specific and therefore effective drug with consequent delay in the patients recovery.This is illustrated by case MI994 a female age 34 suffering from a carbuncle of the chest wall.On admission to hospital she received first aureomycin and then terramycin.(Her pre-admission therapy was not recorded.)The laboratory found the invading organism to be S.aureus (haemolytic) and that it was sensitive only to chloromycetin. After receiving only 8.5 grams of this drug she became well.

Some practitioners did not at any time use the recommended antibiotic as in case MI308. This adult male sustained a laceration of his scalp in a traffic accident.He received dicrysticin from the second day after injury until a total of 26 mls had been administered.A swab was sent to the laboratory three weeks after admission and the organism causing the infection in his wound was identified as S.aureus sensitive only to chloromycetin. The patient never did receive the suggested antibiotic and his wound was still discharging,according to the nursing notes,forty days after admission to the hospital. This patient would certainly have done better if he

had been given the recommended antibiotic.

Another patient M3965 was admitted suffering from a carbuncle of the neck. He was treated with penicillin 10 million units and terramycin 20 grams, without any apparent benefit. The reason was that a swab from the wound showed, in the laboratory the infecting organism to be S.aureus sensitive only to chloromycetin and erythromycin. The patient was operated upon in the classical manner of the pre-antibiotic era and left hospital as might be expected in the usual time of five and a half weeks. One cannot help feeling that this patients infection could have been dealt with more rapidly if he had been given a suitable antibiotic.

There were some instances too in which the laboratory appeared to have been in error naming as the cause of a disease an organism which does not as a rule invade the tissue affected. Case M555 is an example. He was admitted to hospital with lobar pneumonia and diabetes. The laboratory received a sputum specimen five days later. The technicians noted on their report the presence of D.pneumoniae seen on direct examination but these did not grow on culture. The only organism that did show up was Aerobacter and it was insensitive to penicillin. The attending physician had given 800,000 units a day of this drug from the time the patient was admitted. This treatment had without doubt reduced the viability of the D.pneumoniae to the extent that they could not grow on culture. It is apparent that the Aerobacter was a mild secondary invader and it did not interfere with the patients recovery. The report as received by the physician mentioned only the organism

that grew and could be positively identified which was the relatively harmless Aerobacter.The real error was in delaying the submission of a sputum specimen until after treatment had been established.

A similar case was M54 who also suffered from pneumonia and whose sputum specimen was sent for culture some days after treatment with penicillin had been started. Once again only Aerobacter grew on culture and the patient recovered after a weeks treatment with penicillinwhich was active against the real pathogen,probably D.pneumoniae.The laboratory was able to report only the organism which grew and as it was not sensitive to penicillin it appeared to be in error.

In this series of 554 cases only about fifteen per cent of patients recovered in a reasonably short time without receiving the antibiotic found to be effective by in vitro tests. Enough of these fifteen per cent were similar to the two cases mentioned above to question if any pathogenic organism differs greatly in its in vivo response from the in vitro tests as carried out in the Provincial Laboratory.

In this series of cases,there were three times as many staphylococcal infections as infections caused by other organisms. This is set out in Table V overleaf.

As about 60% of staphylococcal strains are resistant to penicillin,25% resistant to aureomycin and terramycin and 17% resistant to chloromycetin,the need for sensitivity tests as an aid to good treatment could not be more clearly indicated.

Physicians sometimes ask concerning the strength of the antibiotic solution on the disc. It should be emphasised that this question was answered in 1951 by an investigation carried out by Fairbrother and Martyn at the Manchester Royal Infirmary (25). They found that the antibiotic concentration on the disc should be almost exactly as used in the Provincial Laboratory and given in table II. A two-fold increase or decrease did not appreciably affect the test but by increasing the potency of the antibiotic beyond this range they found insensitive organisms were shown to be sensitive. The antibiotic concentrations on the disc, therefore, must be adjusted so that zones of inhibition are given only by organisms whose sensitivity are within therapeutic limits. This is particularly important in those instances in which the physician wishes to use one of the broad spectrum antibiotics.

This study suggests a therapeutic procedure which it is felt could be brought into use with benefit to the patient. Those patients suffering from a disease usually caused by a gram positive coccus such as pneumonia septicaemia etc. could be started on penicillin and a specimen submitted at once to the laboratory to confirm the efficacy of this drug or suggest one of the others. Similarly patients suffering from a disease usually caused by a gram negative organism such as pyelitis could be started on a sulphonamide combination such as gantrisin and the laboratory report used as a guide to any change of therapy required.

Such a procedure would lend a degree of precision to treatment and do away with the so-called therapeutic trials which delay recovery and are costly to the patient.

Table V

Number of isolations of various organisms from 334 in-patients at the University of Alberta Hospital Jauuary I to Decenber 3I I953 in order of frequency.

Staph.aureus	I62
E.coli	6I
Strep.haemolyticus	37
P.aeruginosa	26
H.influenzae	25
Aerobacter	22
Strep.viridans	20
Paracolon	I6
D.pneumoniae	I4
Alkaligenes	9
Feacal strep.	9
P.morgani	9
S.albus	9
Micrococci	8
P.vulgaris	5
Cl.welchii	3
K.pneumoniae	2
Anaerobic strep.	I
Diphtheroids	I

Conclusion

As a result of this study it is now believed possible to treat bacterial infections intelligently and in a scientific manner. The disc method of sensitivity testing as carried out in the Provincial Laboratory will ascertain for the practitioner which of the commonly used antibiotics are effective against the microorganism infecting his patient.

It is not considered necessary to determine the exact dilution of the drug required in the plasma as the recommended dosages in common use are adequate and should not as a rule be exceeded.

Sensitivity testing will usually suggest a number of effective antibiotics that may be used with benefit. The choice in such cases is left to the practitioner.

Repeated sensitivity tests are advised as some organisms have the ability to become rapidly resistant to the antibiotic with which they are in contact, making the use of an alternative antimicrobial agent essential for the rapid cure of the patients infection.

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